STROMA AND FORMATION OF PERITHECIA IN HYPOXYLON

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 296

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(WITH PLATE XVIII AND SEVEN FIGURES)

Hypoxylon and its allies have been left more or less uninvestigated on account of the coriaceous structure of the stroma and the difficulty of cutting satisfactory thin sections. DE BARY gave a general summary of FUISTING'S researches on *Hypoxylon* and other members of the family, and said that it agreed with *Xylaria polymorpha* in the structure of stroma and the development of asci. He also stated that there appeared in the young coil that is the primordium of the perithecium "a row of broad cells irregularly rolled up and full of protoplasm," called by FUISTING Woronin hyphae. As the perithecium grows, these disappear by gelatinization, and the ascogenous hyphae, the periphyses, and the paraphyses grow out from a subhymenial layer of 6–8 cells that line the perithecium. The whole ascocarp, according to DE BARY, is filled with a mass of paraphyses before the ascogenous hyphae appear at their base and grow up between them.

In recent years only two of the family Xylariaceae have been studied, *Poronia punctata* by DAWSON,[1] and *Xylaria* by BROWN.[2] In that part of his study related to the development of the perithecium, BROWN says that in the center of a tangle of hyphae smaller than the others there are broad cells shorter and richer in protoplasm. These he identifies as Woronin hyphae, and states that they enlarge and probably divide, and then round off to form the large multinucleate ascogonia which usually fall to the bottom of the perithecium and there bud out the ascogenous hyphae. He further

[1] DAWSON, MARIA, On the biology of *Poronia punctata*. Ann. Botany 14: 245–260. 1900.

[2] BROWN, H. B., Studies in the development of *Xylaria*. Ann. Mycologici 11: 1–13. 1913.

states that paraphyses arise by an increased growth from the cells of the inner perithecial wall. The nuclear program, although not clear, is thought to involve an increase in the number of nuclei from the first uninucleate Woronin hyphae, and probably also further division in the ascogenous hyphae. From the comparative size of the nuclei he inferred fusion in the ascus, and this was the only fusion occurring in the life history.

Material and methods

The material used was collected in September from dead beech bark at Sullivan, Ohio, by Professor CHAMBERLAIN, who noticed that it seemed soft when all the other stromata around it were characteristically hard and mature. Specimens were sent to Mrs. FLORA PATTERSON, mycologist at the Bureau of Plant Industry in Washington, and she identified the form as *Hypoxylon coccineum*. It was fixed in chromoacetic acid and stained in haemotoxylin. Some of the material, which had not been satisfactory in safranin, was destained and then run into haemotoxylin; and some of the very young material was counterstained in gold orange. Both of these latter methods gave good results, because they differentiated the fungus cellulose and outlined the hyphae sharply, yet left the nuclei clear black and sharply marked. The sections were cut 2, 3, 4, 5, 8, and 10 μ thick, the younger stages being cut very thin. Whether or not the late fall development of this stroma is usual is not known to the writer.

Description of stroma

In longitudinal section (fig. 1) the stroma of *Hypoxylon* shows a differentiation into four distinct regions that can be seen in thin sections even with the naked eye. These are (1) an innermost central region, round in shape, which under the microscope is marked by a loose arrangement of hyphae emerging from the substratum; (2) a compacted zone above this of large, parallel, mostly empty hyphae that form a dome over the central region and up the main body of the stroma; (3) the perithecial layer of loosely woven hyphae with much interhyphal space and perithecia scattered throughout the region; and (4) the superficial layer which is further differentiated into a line marking off the fruiting zone by

intertwining hyphae running parallel to the stroma and taking a very dark stain, a space occupied by loose hyphae with dense protoplasmic contents, and a bounding surface of close hyphae staining black and doubtless containing the remnants of conidiophores.

In the central region three types of hyphae are distinguishable. The most conspicuous of these (fig. 2) is the one of large long cells

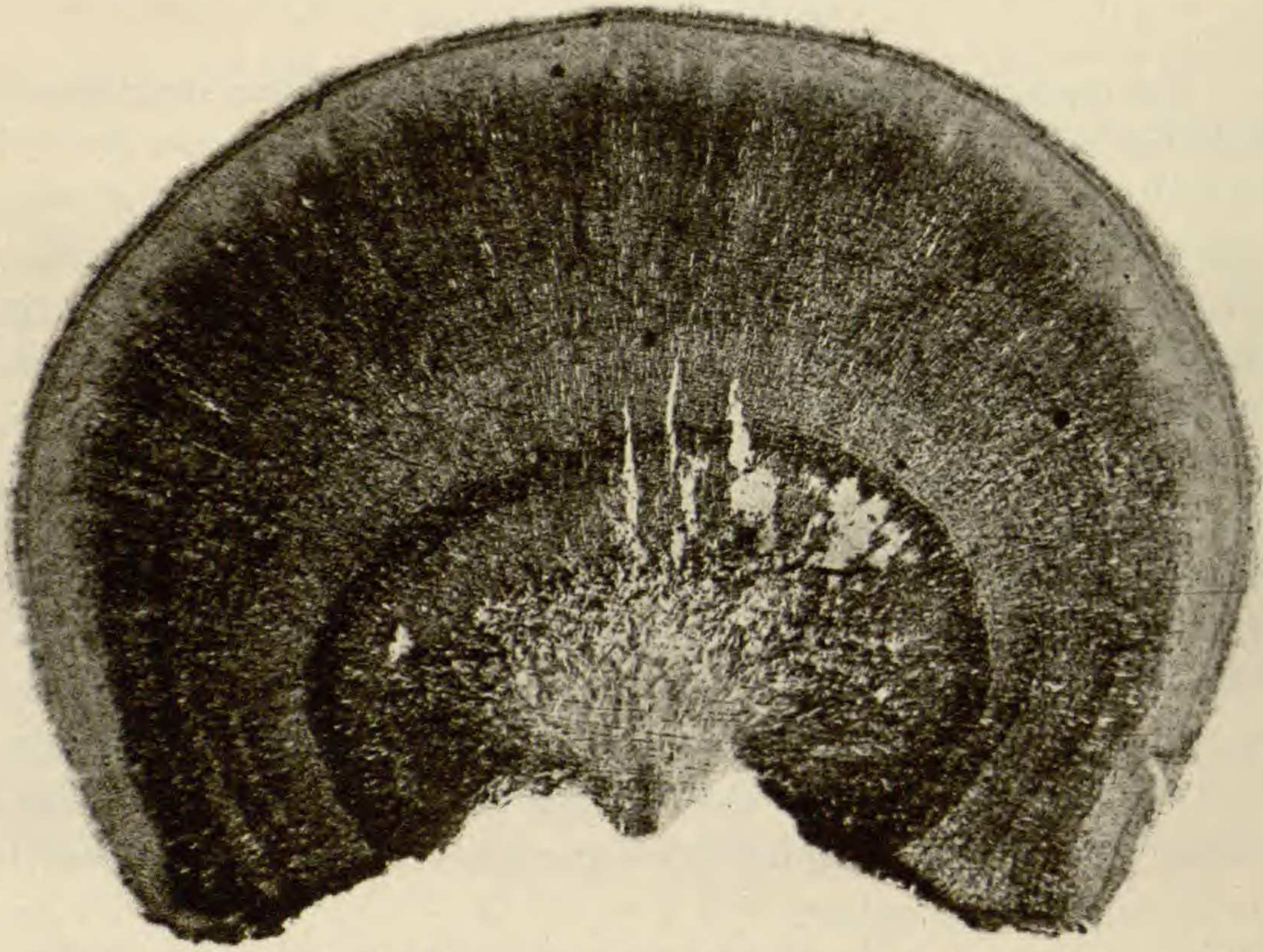
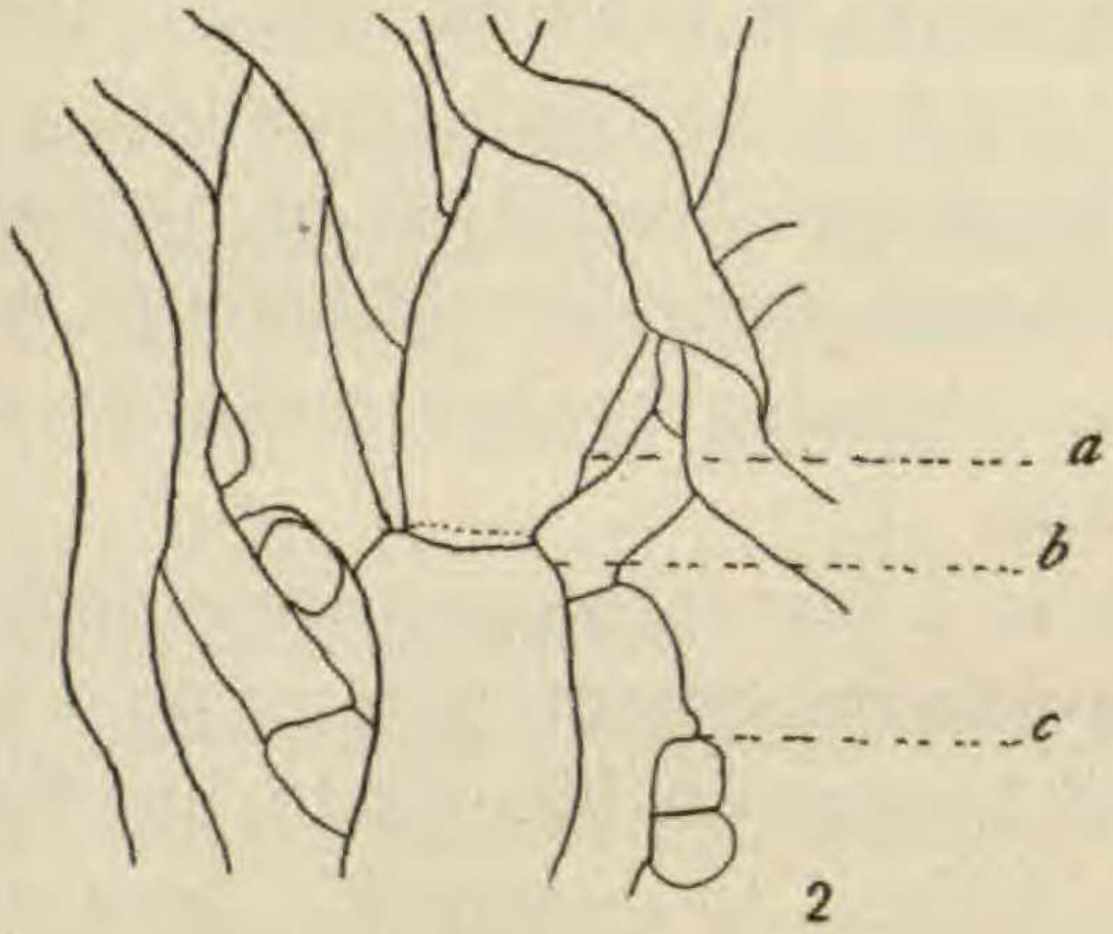


FIG. 1.—Longitudinal section showing borders of the four zones of stroma and the additional markings seen in compact layer of stroma; perithecia visible in fruiting zone; ×26.

staining brown, between which are small hyphae with dense protoplasm, and still smaller ones not staining so densely. These are apparently differentiated from the time of emerging from the substratum into stroma-forming hyphae, into hyphae which form the perithecium, and into those which form the conidiophores and superficial layers. The supporting structure is formed entirely by the largest filaments by means of various devices for interlocking the whole mass and giving to the stroma sufficient firmness, and the same devices are used also in the zone above this. One of the

most frequent devices is that of specially adapted articulation surfaces near the cross-walls (fig. 2*b*), where the cell of another



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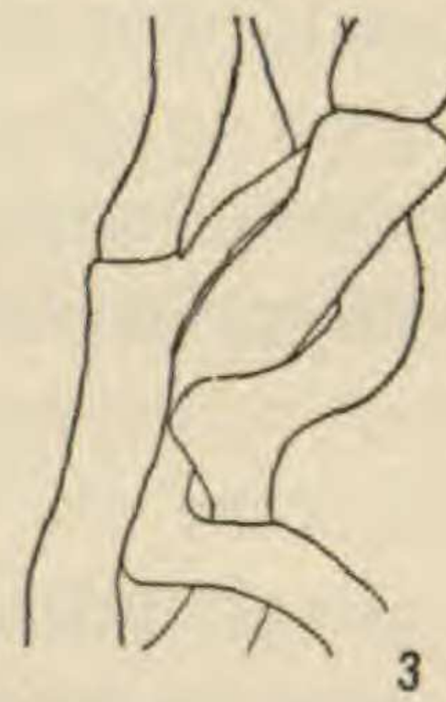

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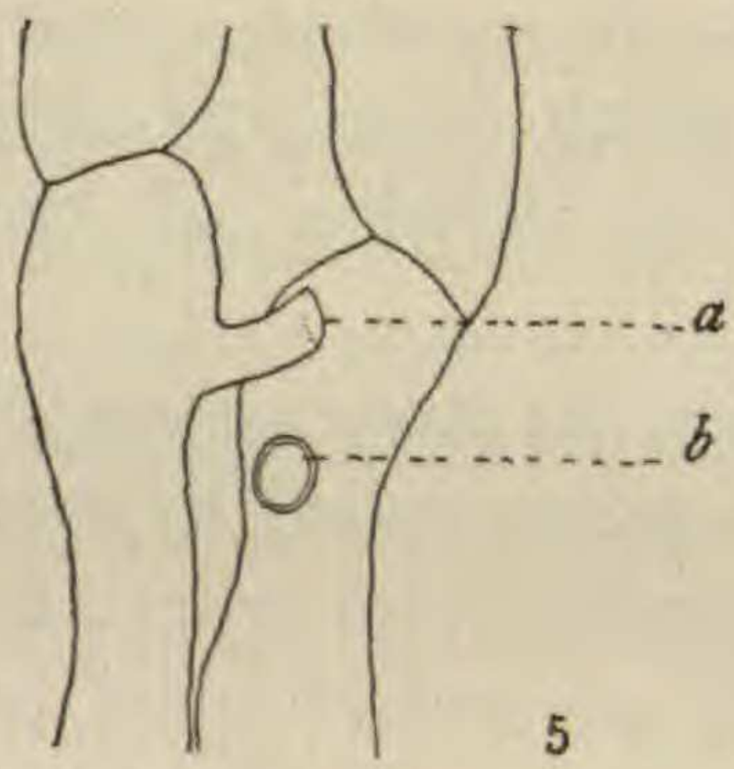

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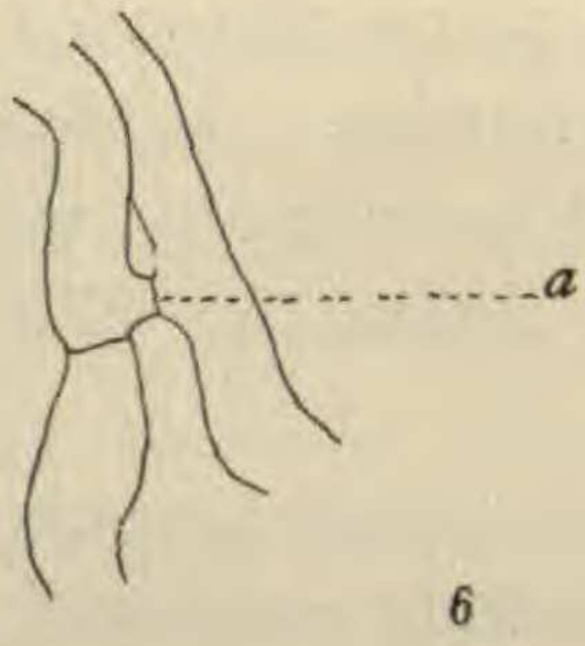

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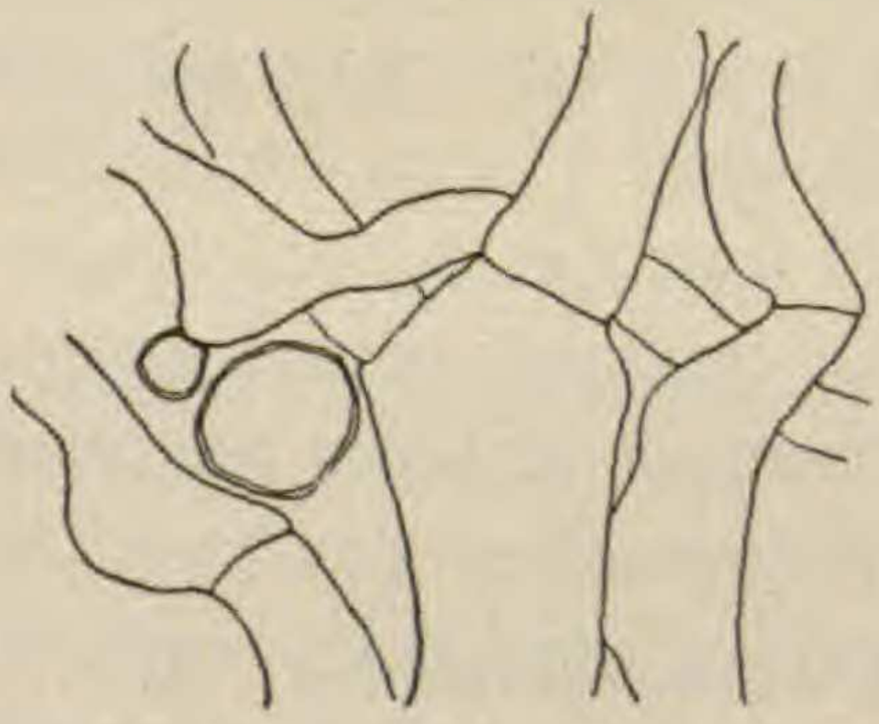

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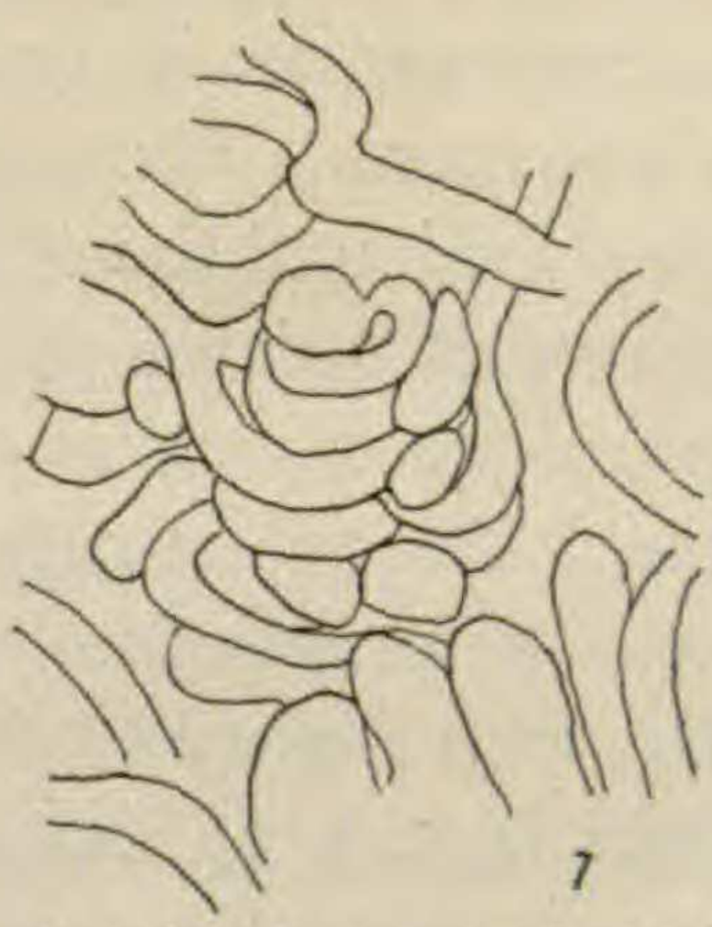

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FIGS. 2–7.—Fig. 2, portion of hyphae from central region showing two principal variations in size and methods of mechanical support; *a*, pit through wall; *b*, articulation surface at septum; *c*, bulbous swellings and depressions fitting together; ×1250; figs. 3, 4, hyphae from same zone showing support gained by twisting and branching; ×1250; figs. 5, 6, interhyphal connections: *a*, bordered hole from sectioning protuberance; ×1250; fig. 7, surface view of early perithecial coil; ×1250.

thread rests against a flat supporting surface for a brace. Another method of support (figs. *2c*, *3*) is the interlocking of adjoining hyphae

by bulbous swellings from one cell fitting into corresponding depressions of the next. Sometimes the fungus thread branches dichotomously and forms a rest for another hypha in the fork (fig. 4), and again the two often intertwine and bend around each other (fig. 3). Contrary to expectation, the walls of the three types of hyphae are uniform in thickness, and although irregular thickenings of cellulose in the walls of the stroma-forming hyphae make them uneven, they are not really thicker. Pits (fig. 1*a*) through the walls are abundant, and although in a few rare cases these seem to be open, in the great majority a definite separating membrane is very clear. The cells of the smaller hyphae are binucleate, but the nuclear condition in the larger is difficult to distinguish, because in the few places where the contents are visible they contain dark-staining granules. As definitely as could be distinguished, however, they have many multinucleate cells which have probably developed from the binucleate condition by division of the nuclei.

In the compact zone above the central region the stroma appears to be uniformly composed of the same large empty-celled hyphae already mentioned, which radiate out in parallel rows from the center to the periphery; but if the filaments are spread apart or a cross-section is examined the same differentiation of the three sizes is seen. The walls show the same thickenings and pits already described, and the cells fit into each other in the same way, making a very firm dense pseudoparenchyma. In some places, beside the incurving of cells to each other, they hold together in the corners, much like xylem cells. One peculiarity of this part of the stroma seen in longitudinal section is the large number of holes bordered by walls (fig. 5*b*). These are cut ends of protuberances from the cells (figs. 5, 6), and are one of the methods of mechanical support not found in the previous zone. They are not the technical clamp connections of DE BARY'S description, because they occur not only near the septa, but from any place along the cell to another in the same hypha, and because they cannot be so named until their method of development has been established. Here they are blind tubes that reach from one cell to fit flat against the wall of an adjacent hypha and clamp the two together.

Other peculiarities of this part are the brown mottled appearance of the whole region, due probably to irregular depositing of some substance in the walls, and the further zonation of this portion of the stroma (fig. 1). This last character is one of the peculiar taxonomic features of *Daldinia*, and in *Hypoxylon* is not evident except through the microscope. These parallel markings are due to irregularities in growth that result in a region of short cells bordering a region of long cells, and to the hyphae intertwining more at this point.

On the outer edge of this portion of the stroma the large hyphae end at varying levels, sometimes with a club-shaped enlargement, and do not give a definite line of demarcation. From between these the two types of hyphae rich in protoplasm pass out to form the upper layers, and of these the larger do not pass much beyond the lower part of the perithecial zone, where some of them form the fruiting bodies, and the smaller continue on beyond this to form the three superficial boundaries. It is this situation which indicates that the sizes of these hyphae were differentiations according to function and not accidental variations. In this region of scattered hyphae that form the perithecial layer, and also in the three outer regions, the cells are typically binucleate, although a very few with several nuclei are seen; and this is true also for the hyphae forming the perithecial wall. On the outer surface of the stroma are spherical excrescences like bubbles, and these are related to the interhyphal spaces. They are probably the excretion of some oily substance through the stroma, and it is owing to this that the young stromata feel smooth and slightly greasy to touch.

Formation of perithecia and ascogonia

In the formation of perithecia in *Hypoxylon* the first evidence of their origin is the coiling of hyphal ends or of branches. As already stated, it is the larger of the two protoplasm-filled hyphae differentiated from the substratum that do this, and apparently they do not show any increase in size before or immediately after this stage. Other hyphae of the same size surround these initial coils and form a small circular knot (figs. 7–9), which was the earliest stage BROWN recognized in the development of the perithecia in

Xylaria. From the evidence there is no basis for believing in any earlier differentiation of Woronin hyphae as initiating the coiling process; but they do become differentiated in the center of the coil by an increase in size very soon after the perithecial primordia are well started (fig. 10). Growth in the size of the perithecium is accomplished by increase in the length of the wall hyphae, and also by the addition of other hyphae around the outside. With this increase in size the wall layers become thinner and compact, and some of the inner hyphae decrease in size and become absorbed, probably furnishing nourishment for the fertile branches (figs. 10, 11*d*). A large number of perithecia start but few mature, and these, logically in relation to their food supply, are mostly toward the inner line of the perithecial zone. The others remain intact and are scattered throughout the fruiting region, apparently inhibited from further growth at any stage in their development, and remaining without change at that stage. As an exception to this, some of the larger perithecia that have reached the point where they contain ascogonia and then become abortive show signs of deliquescing and disintegrating. As a rule the older perithecia are toward the top of the stroma and the younger stages are down toward the substratum.

Within the perithecium the filament in the center develops into the Woronin hypha (fig. 10). It increases in length, and in size and number of cells; and after this some of the cells round out, increase in size, and eventually separate from each other to form the ascogonia. The enlargement is not uniform as to the size and shape attained, for these ascogonia (fig. 11*b*) are many of them uneven and contorted in outline, and many of them retain narrow stalklike connections with cells from which they have not become completely separated.

The nuclear condition in the Woronin hyphae and ascogonia is the critical point, and is hard to determine because of the extreme variations in the size of the nuclei and the difficulty of determining successive stages. As said before, the cells of the Woronin hyphae are binucleate (fig. 9), and as they enlarge (fig. 10) they show a steady and marked increase in the size of the nuclei from the time the knot is well formed. There are one or two divisions, after

which they increase their size, and at the time of the rounding out of the ascogonia there are four, sometimes more, large nuclei five or six times the size of the originals. As the ascogonia increase to the enormous odd shapes found in the more mature perithecia, the nuclei show great variation in size and number (fig. 11). During this stage they undergo rapid division without maintaining their size, and in the ascogonia which show evidences of budding out the ascogenous hyphae they are small and number sixteen or more in a section. Evidence for this interpretation of the nuclear program is gained also from the perithecial wall, because as it grows more mature in character these changes in size and number are unmistakable. There is no evidence of fusion in any stage of the development of the ascogonia. No mitotic figures were visible, but in many cases the position of the two nuclei was such as to suggest late division.

The ascogonia do not drop to the bottom of the perithecium before germinating. Instead the increase in size of the wall is toward the periphery of the stroma, and the ascogonia, although in the same actual position, seem to have dropped because of the expansion of the perithecial wall toward the surface.

Ascogenous hyphae

The material used was just at the stage when the ascogenous hyphae were beginning to bud out, and no detail about the formation of these can be given. Great care was necessary not to confuse some of the stalk connections where separation was incomplete with ascogenous hyphae. These could be recognized by their direction and evident connection with some part of the filament traced through serial sections. Legitimate cases of buds (figs. 12, 13) just formed were found, however, and although of course the nuclear situation, separation, and branching could not be determined, apparently the procedure would be the same as described for other forms where the nuclei migrate into the buds from the ascogonia, and further division takes place there.

A supposition lacking evidence to support it is that the paraphyses described by DE BARY and BROWN might be formed from these early ascogenous hyphae or branches of them instead of

from a subhymenial layer. This seems reasonable, since at this stage the compact wall of the perithecium is an unlikely place for new growth, and there is no evidence of new hyphae being found to account for the paraphyses in other forms. It seems very probable that this is their origin here.

Summary

1. The stroma is differentiated into four regions, and in one of these further zonation is evident.

2. The firm structure of the stroma is gained by various mechanical devices for support, such as tubular extensions from cells, branching and intertwining of hyphae, and special articulation surfaces.

3. The hyphae are differentiated into three types from the time of their emergence from the substratum: those that form the major part of the stroma, those that form the perithecia and Woronin hyphae, and those that form the superficial layers and probably the conidiophores.

4. The cells of the hyphae are originally binucleate, but may become multinucleate.

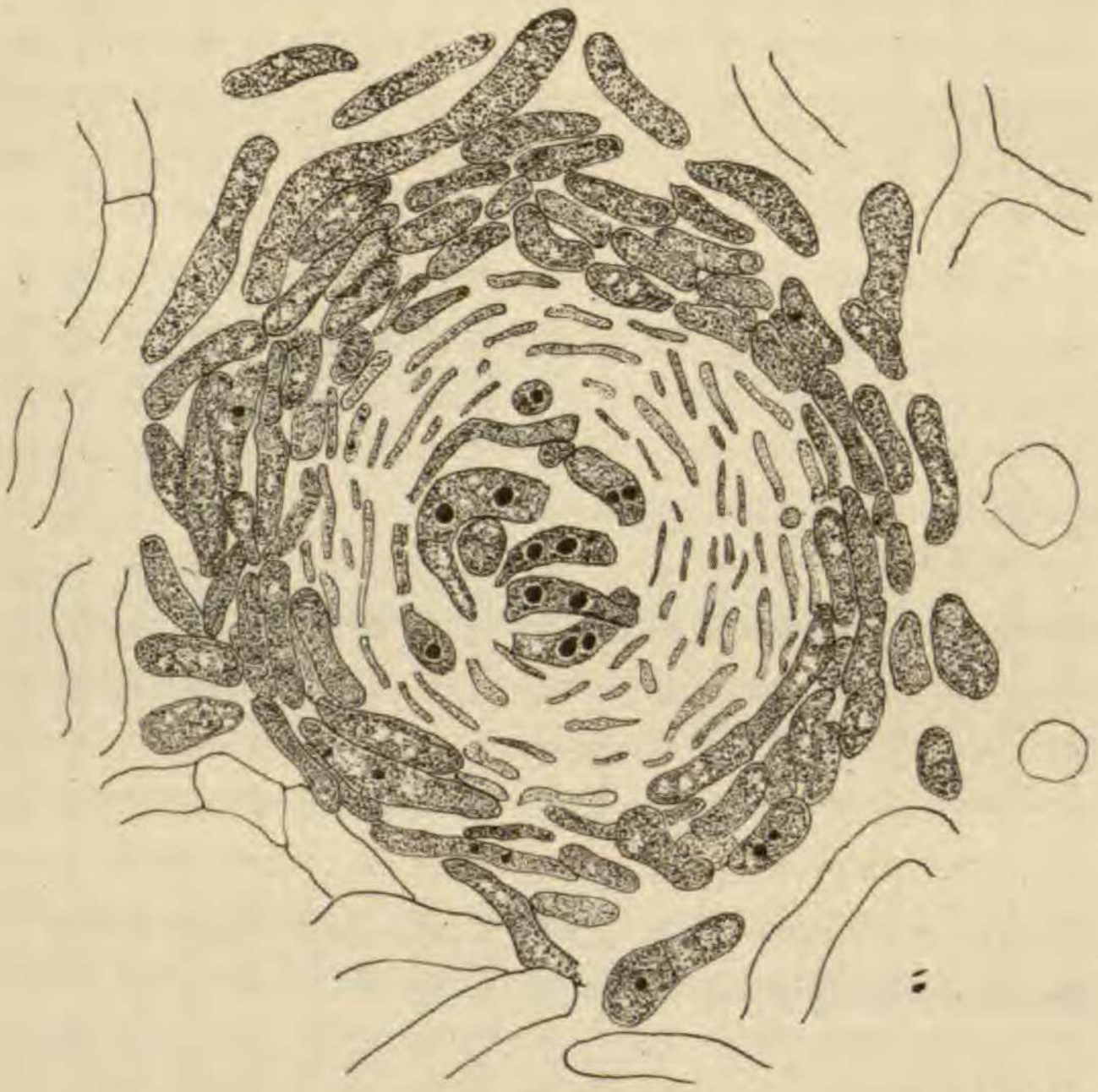
5. The formation of the perithecia is initiated by the massing of the hyphae into a circular knot, within the center of which the Woronin hyphae differentiate.

6. The ascogonia develop from the cells of the Woronin hyphae by rounding out, partially separating from each other, and increasing in size.

7. The ascogonia do not drop to the bottom of the perithecium in the older stages, but come to lie comparatively closer to the bottom by an expansion of the perithecial wall toward the periphery of the stroma.

8. The nuclear program within the ascogonia is one of few divisions and great increase in size, up to the stage where the ascogonia are well rounded out, and then of rapid division without the maintenance of size.

9. The ascogonium buds out protuberances that are the beginnings of the ascogenous hyphae.



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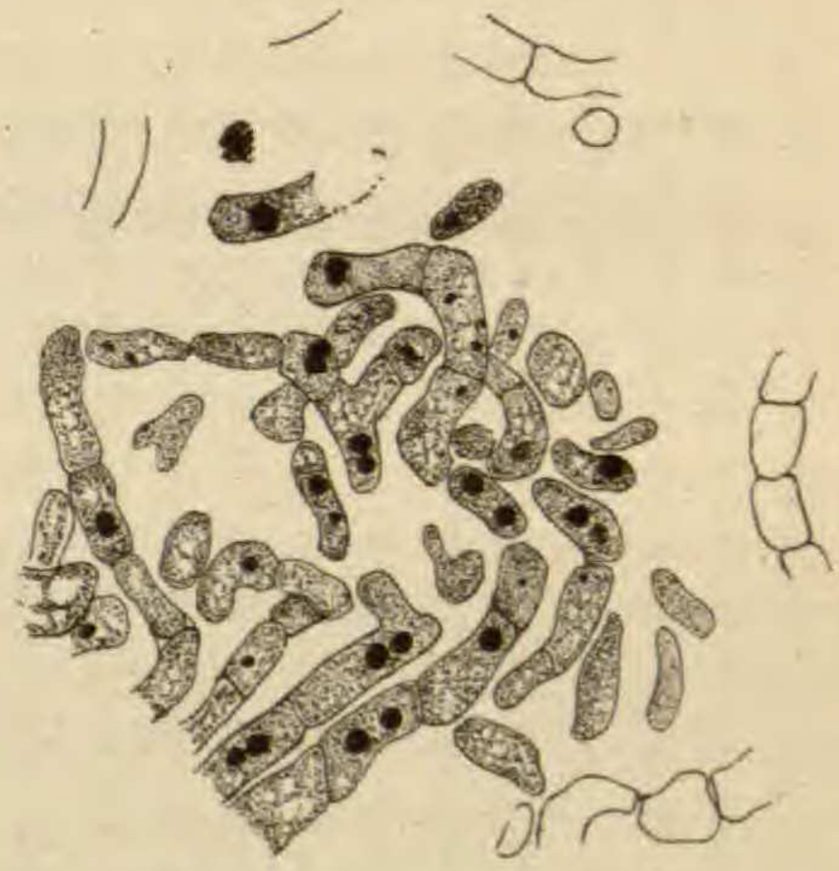

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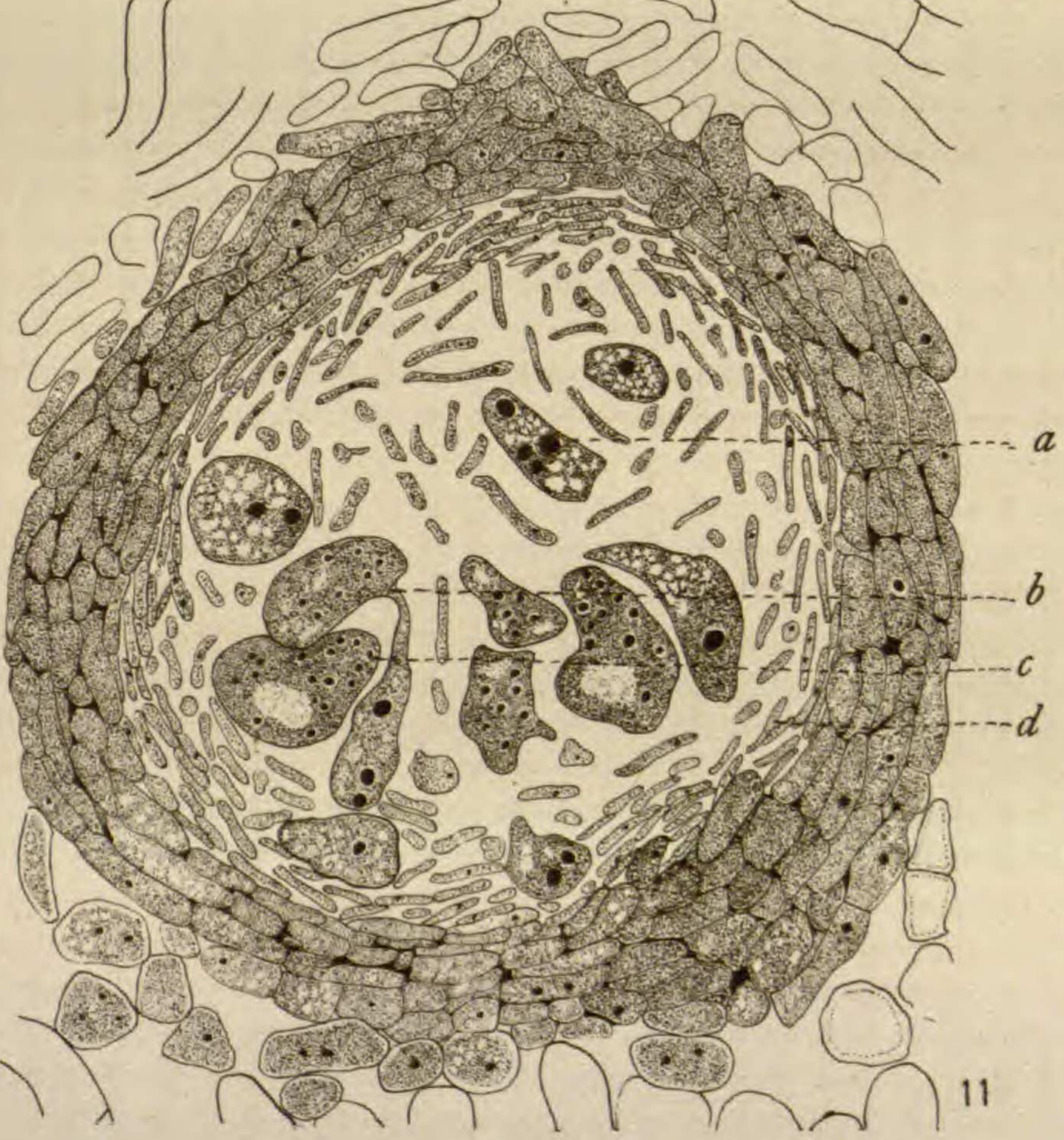

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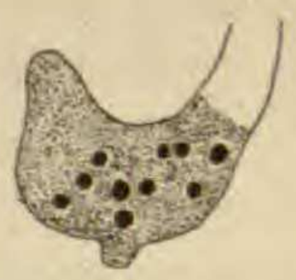

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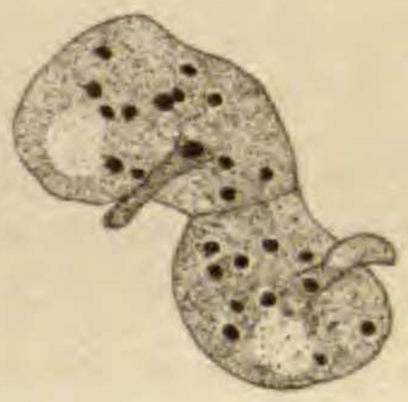

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LUPO on HYPOXYLON